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Studies on *Passiflora incarnata* Dry Extract. I. Isolation of Maltol and Pharmacological Action of Maltol and Ethyl Maltol

Nobuo Aoyagi,[1a] Ryohei Kimura, and Toshiro Murata[1b]

National Institute of Hygienic Sciences[1a] and Shizuoka College of Pharmacy[1b]

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The compositions of the fractionized samples of *Passiflora incarnata* dry extract were investigated. Harmine was found in the ether soluble fraction by paper and thin-layer chromatographies. Maltol was isolated from the 2 N HCl soluble fraction.

The pharmacological properties of *Passiflora incarnata* dry extract, the 2 N HCl soluble fraction, maltol and ethyl maltol were investigated. The 2 N HCl soluble fraction decreased the oxygene uptake by rat brain cortex slices at a high concentration.

Maltol caused a depression in mice and showed potentiation on hexobarbital induced sleep, anticonvulsant action with such high doses as toxic, and inhibition on the spontaneous motor activity in mice with low doses. Ethyl maltol showed similar actions but more potent anticonvulsant avtivity and less potent inhibitory effects on the spontaneous motor activity.

Since earlier in 20 th century *Passiflora incarnata* (abbreviate as PI) has been used as a medicinal plant for neurosis in Europe. As the adverse reactions of synthetic tranquilizers have been getting into clinical problems, we are interested in some crude drugs having a depressant action such as *Passiflora* and *Valeriana etc.* PI is cultivated in open field and in a green-house nowadays, and some commercial preparations mixed with other crude drugs have been available as a sedative.

Many works[2–8] have been carried out on the alkaloids contained in PI. Neu[2] isolated harman from PI and identified it with passiflorin which had been previously described by Peckolt.[3] Lutomski[4c] isolated harman, harmol, harmaline and unidentified substances, A and B, from the leaves and stalks of PI. Hultin[6] found harman, harmaline, harmalol and harmol in the plant. Bennati[7] confirmed the presence of harman, harmaline and harmine in PI. Pethke, *et al.*[8] found only harman (1.2—3 ppm) and could not detect other harmala alkaloids in PI. As the ingredients other than the alkaloids some flavone-C-glycosides were isolated from PI.[9]

On the pharmacological properties of PI preparations, Lutomski[10] reported that both of the alkaloids and flavonoids had a sedative action. On the other hand harmala alkaloids such as harmine and harmaline are generally known to inhibit monoamine oxidase[11] and have

1) Location: *a) Kamiyoga 1-18-1, Setagaya-ku, Tokyo; b) Oshika 2-2-1, Shizuoka-shi, Shizuoka.*
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3) Th. Peckolt, *Ber. Dtsh. Pharmaz. Ges.*, **19**, 343 (1909).
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been used as a central stimulant, especially as a tremorigenic agent.[12] These results suggest that the alkaloid fraction of PI might contain pharmacologically active components having depressant action which antagonize the stimulant action of harmala alkaloids.

In this paper PI dry extract (abbreviate as PIE), a commercial preparation, was studied on the pharmacological properties and the isolation of sedative components from it was attempted by the authors.

Material and Method

Materials——PIE was kindly provided by Paul Knufunke G.m.b.H. Maltol and ethyl maltol were kindly provided by Takasago Koryo Co. and were used by dissolving in 15—30% propyleneglycol for pharmacological assays. Harmine and harmaline were obtained from commercial source. Harmol (15 mg) was synthesized by hydrolysis of harmine (20 mg) in 6 N HCl.[13] mp 318—320°. *Anal.* Calcd. for $C_{12}H_{10}ON_2$: C, 72.71; H, 5.09; N, 14.13. Found: C, 72.44; H, 5.10; N, 14.01. UV λ_{max}^{EtOH} nm: 242, 304. IR ν_{max}^{KBr} cm^{-1}: 3260 (OH), 1635.

Animals——ddY strain male mice, weighing 15—18 g, and Wistar male rats, weighing 160—180 g, were used.

Fractionation of PIE——PIE (100 g) was suspended in 200 ml of 28% ammonia solution and extracted with 1000 ml of CH_2Cl_2. The CH_2Cl_2 solution was dried over anhydrous Na_2SO_4 and evaporated to dryness. Dark brown residue (0.23 g, CH_2Cl_2 soluble fraction) was obtained. The residual aqueous layer was adjusted to pH 13 with 2 N NaOH and extracted with 1000 ml of ether. The ether solution was dried over anhydrous Na_2SO_4 and concentrated to dryness. Brown residue (0.015 g, ether soluble fraction) was obtained. On the other hand the another portion of PIE (100 g) was suspended in 28% ammonia solution and extracted with 1000 ml of CH_2Cl_2. The CH_2Cl_2 solution was then extracted with 200 ml of 2 N HCl. The acidic solution was neutralized with $NaHCO_3$ (pH 7—8) and extracted with 300 ml of CH_2Cl_2. The CH_2Cl_2 solution was concentrated to dryness. Brownish red residue (0.17 g, 2 N HCl soluble fraction) was obtained.

Isolation of Maltol——Maltol (0.05 g of colorless needles) was obtained from the 2 N HCl soluble fraction (0.17 g) of PIE by sublimation at 60° under reduced pressure and was identified with an authentic sample by IR spectrum and admixture. mp 161—163°. *Anal.* Calcd. for $C_6H_6O_3$: C, 57.14; H, 4.89. Found: C, 57.25; H, 4.80. UV λ_{max}^{EtOH} nm (log ε): 278 (3.91). IR ν_{max}^{KBr} cm^{-1}: 3230 (OH), 1650, 1615, 1555. Mass Spectrum *m/e*: 126 (M^+).

Paper Chromatography (PC)——PC was carried out on Toyo Roshi No. 51 paper. The solvent systems were (1) *n*-butanol–AcOH–H_2O (5 : 1 : 4) and (2) benzene–CCl_4–MeOH (1 : 1 : 1). The each chromatogram was detected under UV light and by spraying Dragendorff reagent.

Thin-Layer Chromatography (TLC)——TLC was carried out with thin-plates (Wakogel B-5, 0.25 mm thick) using MeOH as developing solvent. The spots were detected as described in PC.

Pharmacological Assay——a) Acute Toxicity and Gross Behavior Observation: Test drugs were injected subcutaneously in mice and the acute toxicities were determined by the observation for 48 hr. LD_{50} was calculated by the up and down method. After the drug administration changes in gross behavior of mice were observed according to the check list of Irwin.[14]

b) Oxygene Uptake by Rat Brain Cortex Slices: The estimation of oxygene uptake by rat brain cortex slices was carried out using Warburg manometric apparatus according to the conventional method.[15] Brain cortex slices (average wet weight 70—80 mg) were prepared from a rat and placed in an ice-cold manometric vessel which contained an incubation medium and test sample. The composition of the medium was as follows. NaCl, 128.2 mM, KCl, 5.13 mM (105 mM as to K^+ effects[16]); $CaCl_2$, 2.75 mM; $MgSO_4$, 1.28 mM; glucose, 10 mM; Na_2HPO_4, 10 mM (brought to a pH 7.4 with 1 N HCl).

c) Spontaneous Motor Activity: Alterations in spontaneous motor activity in mice were determined using an activity monitor (TN type, Natume Seisakusho). Thirty minutes after subcutaneous injection of test compounds, mice were placed in the counting box. Control animals received only the vehicle. The number of times blocking the light beam was recorded for 90 minutes.

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d) Hexobarbital Sleeping Time: Thirty minutes after subcutaneous injection of test compounds or ninety minutes after oral administration of maltol, a dose of 100 mg/kg of sodium hexobarbital was injected intraperitoneally in mice. A loss of righting reflex was employed as an index of sleep.

e) Pentylenetetrazole Convulsion: Drugs were injected subcutaneously in each group of 10 mice 30 minutes prior to intraperitoneal administration of 150 mg/kg of pentylenetetrazole. The intervals from pentylenetetrazole injection to manifest clonic convulsion, tonic convulsion and death were recorded. Meprobamate was used as a reference drug.

f) Strychnine Convulsion: Drugs were injected subcutaneously in mice 30 minutes prior to intraperitoneal administration of 1.5 mg/kg of strychnine nitrate and the number of the animals produced convulsion or died was recorded for 2 hr. Meprobamate was used as a reference drug.

Result

Paper and Thin-Layer Chromatographies of the Fractionized Samples of PIE

As Table I shows, the chromatograms of the ether soluble fraction revealed a spot having the same *Rf* and the same color of fluorescence as that of harmine. The several other spots were also found on the chromatograms under UV light, but not detected with Dragendorff reagent. On the other hand, any harmala alkaloids were not identified on the chromatograms of the 2N HCl soluble fraction because of tailing.

Table I. *Rf* Values of the Sample and Harmala Alkaloids

	Solvent	Ether SF[a)]	Harmine	Harmol	Harmaline
PPC	1	0.69 (violet)	0.71 (violet)	0.71 (violet)	0.65 (green)
	2	0.76	0.75	0.61	0.45
TLC	3	0.78	0.77		0.20

a) ether soluble fraction
The parentheses indicate the color of fluorescence under UV light.
The spots were detected under UV light and then with Dragendorff reagent.
solvent 1: *n*-butanol–AcOH–H_2O (5:1:4)
solvent 2: benzene–CCl_4–MeOH (1:1:1)
solvent 3: MeOH

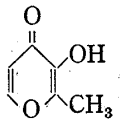

Fig. 1. Structure of Maltol

Isolation of Maltol

Maltol (3-hydroxy-2-methyl-4-pyrone, Fig. 1) was isolated from the 2N HCl soluble fraction by sublimation under reduced pressure. The yield of maltol from PIE was about 0.05%.

Acute Toxicity and Gross Behavior in Mice

Intraperitoneal administration of 500 mg/kg of PIE caused no significant changes in behavior of mice, but 250 mg/kg of the 2N HCl soluble fraction caused decreases in spontaneous activity, respiratory rate and heart rate. The fraction caused tremor-like symptom followed by death in mice with 1000 mg/kg.

Subcutaneous injection of 400 mg/kg of maltol and ethyl maltol produced a decrease in spontaneous activity, bradycardia, hypothermia, relaxation of skeletal muscle, and diminutions of pinna reflex, corneal reflex and ipsilateral flexor reflex. Ethyl maltol caused a loss of righting reflex in all mice with 700 mg/kg while maltol caused a loss of the reflex with a higher dose than that of ethyl maltol. The LD_{50} values of the both compounds were shown in Table II.

Oxygene Uptake by Rat Brain Cortex Slices

As Table III shows, PIE had no effects on the brain respiration at a concentration of 1 mg/ml, but showed 9% inhibition at 3 mg/ml in the presence of K^+ effects. The CH_2Cl_2 soluble fraction remarkably decreased the oxygene uptake at 2 mg/ml though the effect was

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Table II. Acute Toxicities of Maltol and Ethyl Maltol

Drugs	Route	No. of mice	LD_{50} (mg/kg)
Maltol	*s.c.*	10	820
Ethyl maltol	*s.c.*	10	910

Table III. Effects of the Test Drugs on Oxygene Uptake by Rat Brain Cortex Slices

Drug	Conc.	No. of expl.	Conc. of K^+ 5 mM Qo_2 (μl/g/hr)	5 mM Effects	105 mM Qo_2 (μl/g/hr)	105 mM Effects
Control		8	1839±185[a]		2379±183	
PIE	1.0 mg/ml	8	1860±151	+1%	2292±277	−3%
Control		3	2044±100		2496±155	
PIE	3.0 mg/ml	3	1865± 22	−9%	2274±124[b]	−9%
Control		5	1873±124		2333±317	
CH_2Cl_2 *SF*[c]	0.2 mg/ml	3	1836±106	−2%	2109±183	−11%
	2.0 mg/ml	5	594±116[d]	−68%	817±100[d]	−65%
Control		3	2028± 42		2780±177	
2 N-HCl *SF*[c]	2.0 mg/ml	3	503±135[d]	−73%	625±132[d]	−78%
Control		3	2025±107		2637± 58	
Maltol	1.0 mM	3	1950± 93	−4%	2661± 91	+1%
Ethyl maltol	1.0 mM	3	1940±153	−4%	2706±154	+3%

a) mean value±standard deviation
b) significant $p<0.05$
c) *SF*: soluble fraction
d) significant $p<0.01$

not significant at 0.2 mg/ml. More marked inhibition was observed with 2 mg/ml of the 2 N HCl soluble fraction.

Maltol and ethyl maltol had no effects at a concentration of 1 mM.

Spontaneous Motor Activity

As Fig. 2 shows, the spontaneous motor activity was depressed by administration of 75 mg/kg of maltol (approximately 50% inhibition at the period of 60 minutes after administration) and more marked inhibition was observed with higher doses. Ethyl maltol also showed inhibitory effects but less potent than maltol.

Hexobarbital Sleeping Time

As Table IV shows, subcutaneous administration of 300 mg/kg of both compounds prolonged the hexobarbital sleeping time in mice. Oral administration of 300 mg/kg of maltol also showed potentiating effects.

Pentylenetetrazole Convulsion

Pentylenetetrazole (150 mg/kg) caused clonic convulsion followed by tonic convulsion and

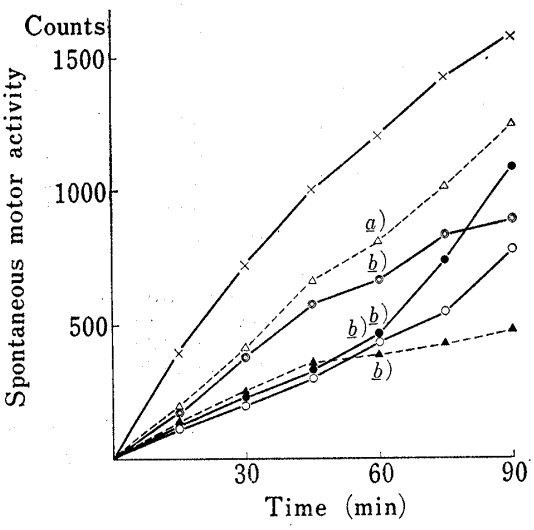

Fig. 2. Effects of Maltol and Ethyl Maltol on Spontaneous Activity in Mice

—×—: control (n=15)
—◎—: maltol (n= 7) 75 mg/kg
—○—: (n= 6) 150 mg/kg
—●—: (n= 6) 300 mg/kg
---△---: ethyl maltol (n= 5) 150 mg/kg
---▲---: (n= 6) 300 mg/kg
n in the parentheses indicates numbers of the experiments.
a) significant $p<0.05$
b) significant $p<0.01$

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death in mice. Ethyl maltol significantly showed antipentylenetetrazole action at a dose of 300 mg/kg, while maltol showed the same effects at a dose of 500 mg/kg (Table V). Meprobamate completely protected mice against pentylenetetrazole convulsion.

Strychnine Convulsion

As Table VI shows, strychine nitrate (1.5 mg/kg) caused tonic convulsion followed by death in 90% of the mice of control group. Ethyl maltol showed antistrychnine action with

Table IV. Effects of Maltol and Ethyl Maltol on Hexobarbital Induced Sleeping Time

Drugs	Dose (mg/kg)	Route	No. of mice	Sleeping time (min)
Control		*s.c.*	10	71± 17[a]
Maltol	300	*s.c.*	10	122± 23[b]
	500	*s.c.*	10	154± 56[b]
Control		*p.o.*	7	96± 17
Maltol	300	*p.o.*	9	134± 37[c]
	500	*p.o.*	7	182± 33[b]
Control		*s.c.*	8	108± 33
Ethyl maltol	300	*s.c.*	8	190± 53[b]
	500	*s.c.*	8	279±113[b]

sodium hexobarbital 100 mg/kg *i.p.*
a) mean value±standard deviation
b) significant $p < 0.01$
c) significant $p < 0.05$

Table V. Effects of Maltol and Ethyl Maltol on Pentylenetetrazole Convulsion in Mice

Drugs	Dose (mg/kg)	Time (min)		
		Clonic convulsion	Tonic convulsion	Death
Control		1.5± 5.5[a]	5.4± 5.5	17±22
Maltol	300	3.3± 3.3	6.1± 4.9	24±38
	500	12.0± 9.2[b]	14.8± 9.9[c]	77±45[b]
Ethyl maltol	300	27.3±13.5[b]	30.7±12.6[b]	40±25[c]
	500	119.6±36.9[b]	120.4±37.3[b]	121±37[b]
Meprobamate	100	—	—	—

pentylenetetrazole 150 mg/kg *i.p.*
a) mean value±standard deviation
b) significant $p < 0.01$
c) significant $p < 0.05$

Table VI. Effects of Maltol and Ethyl Maltol on Strychnine Convulsion in Mice

Drugs	Dose (mg/kg)	No. of mice	No. of tonic convulsion	No. of death
Control		20	18	18
Maltol	300	10	8	7
	500	10	1[a]	0[a]
Ethyl maltol	150	10	10	9
	300	10	3[a]	2[a]
	500	10	0[a]	0[a]
Meprobamate	100	10	10	5[a]

strychnine nitrate 1.5 mg/kg *i.p.*
a) significant $p < 0.01$

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a dose of 300 mg/kg, but maltol did not with that dose though showed remarkable action with 500 mg/kg. Meprobamate had no anticonvulsant action but prevented a half of the mice against death with a dose of 100 mg/kg.

Discussion

The 2N HCl soluble fraction of PIE caused a depression in mice as reported by Lutomski, *et al.*[10] and decreased the oxygene uptake by rat brain cortex slices at high concentrations. From these observations it can be suggested that the PIE or its 2N HCl soluble fraction might contain some sedative components and their depressant effects mask the stimulant effects of the harmala alkaloids.

Maltol isolated from the 2N HCl soluble fraction caused a depression in mice and showed potentiating effects on hexobarbital induced sleep. Maltol also showed anticonvulsant actions on chemically induced seizures with such high doses as toxic, and decreased the spontaneous motor activity in mice with relatively low doses (approximately 1/10 of the LD_{50}). On the other hand ethyl maltol, ethyl substituent compound of maltol, showed similar actions but more potent anticonvulsant activity and less inhibitory effects on the spontaneous motor activity than maltol. These findings suggest that the pharmacological properties of maltol derivatives must be changed by altering the alkyl group in maltol.

It seems difficult to explain that the sedative effects of PIE are only due to maltol which was isolated from PIE by the present authors. Further investigations of the other ingredients of PIE are now in progress in our laboratory.

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